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TITLE: Extracellular Matrix Biomarkers for Diagnosis, Prognosis, Imaging, and Targeting

PRINCIPAL INVESTIGATOR: Richard Hynes

CONTRACTING ORGANIZATION: Massachusetts Institute of Technology
Cambridge, MA 02139-4301

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6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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14. ABSTRACT
The goal of this project is to characterize the extracellular matrix (ECM) microenvironment of mammary tumors and their metastases. The ECM provides multiple cues to both tumor and normal cells that affect their proliferation, survival, migration, invasion and resistance to chemo- and radio-therapy. As such, the ECM is an important component of tumors but has been difficult to analyze until recently. We have defined ECM biomarkers associated with (and in some cases causal of) metastasis. Under this project we are defining the composition of the ECM of human patient tumors, both primaries and metastases, as well as human tumors growing in mice (so-called PDX models). Defining the differences between tumors and in metastases, we seek to develop novel ECM biomarkers and antibodies to them for use in diagnosis, prognosis, early detection, in situ imaging and, eventually, targeting of breast cancer metastases, which are the major cause of deaths from this disease. We expect that the novel approaches that we are using will reveal novel biomarkers and provide sorely needed new approaches to the management and treatment of metastatic breast cancer.

15. SUBJECT TERMS
Breast Cancer, Metastasis, Extracellular Matrix, Tumor Microenvironment, Tumor Heterogeneity

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1. INTRODUCTION:

Specific Aims:

1. Characterize, by novel proteomic methods, the ECMs of mammary tumors from mouse models, xenotransplants of human tumors into mice and human patient samples. Comparisons among these data sets and published data will define novel ECM signatures for tumors of different stages and outcomes.
2. We will then generate recombinant antibodies to these ECM signature proteins.
3. These antibodies will be used to develop diagnostic and prognostic assays.
4. They will also be developed as highly selective imaging reagents to image tumors in vivo, first in xenotransplant models and, subsequently, in patients.
5. The antibodies will also be used to deliver agents (toxins, chemotherapeutics, isotopes and immune modulators) to tumors in vivo, to develop highly targeted therapies for metastases.

2. KEYWORDS:

Breast Cancer, Metastasis, Extracellular Matrix, Tumor Microenvironment, Tumor Heterogeneity

3. ACCOMPLISHMENTS:

HYNES lab:

Major Task 1: Proteomic characterization of ECM in mammary carcinoma progression & metastasis using xenotransplants of human tumor samples in mice.
Major Task 2: Proteomic characterization of ECM from human patient tumor samples
Major Task 3: Investigation of functional roles of ECM proteins that change – in mouse models
Major Task 4: Years 2-4 Development of diagnostic and prognostic ECM biomarker signatures

Major Task 5. Years 4/5
Use of antibodies against ECM biomarkers to image occult metastases and tumors
Major Task 6. Year 5
Use of antibodies to target therapeutic agents to occult metastases and tumors

POLYAK lab: Major Goals for Year 1

1. Perform xenograft assays in immunodeficient mice using the intratumor clonal heterogeneity models of breast cancer developed in our lab and also patient-derived xenografts.
2. Collect primary and metastatic lesions from the resulting tumors, as well as lesions from breast cancer patients and provide these tissues to Dr. Hynes for ECM analysis.
3. Once ECM proteins differences between primary and metastatic or among different metastatic lesions are validated, we will downregulate/overexpress these genes in the same models to determine their consequence on metastasis formation and therapeutic responses.

What was accomplished under these goals?

1 & 2) Major Activities and Specific Objectives:

Hynes lab:
We have been refining our **ECM proteomics** methods to exploit the improved mass spectrometers now available and newer methods for quantitative proteomic comparisons as well as exploring modifications to our ECM enrichment protocols. We have applied these methods to analyses of **PDX models of breast cancer** provided by the Polyak laboratory as well as **human breast cancer patient samples**. We have explored the functions of one of the ECM proteins we earlier showed promotes mammary carcinoma metastases by generating a **knockout mouse line** and investigating its phenotype. We have generated samples of mammary **metastases to multiple different sites** in xenotransplant models for proteomic analyses to investigate the differential properties of the metastastic niches in those different tissues. We have made significant progress on **validation and generation of antibodies** for further investigations of ECM proteins of interest for their role in metastases and their potential for diagnostic, prognostic and therapeutic applications.

Polyak lab:
We have been collecting **fresh breast tumor samples** and using these to generate new **patient-derived xenograft (PDX) models with different metastatic capacities** in NOG mice. We have been also expanding these PDX models to generate sufficient material for ECM studies. We have expanded and collected primary and metastatic lesions from several of our **cell line models of metastatic breast cancer**. We have characterized these tumors for molecular markers to be able to correlate these features with findings from ECM analyses. We provided tissue samples to the Hynes lab from breast cancer patients and from the xenograft models in mice for ECM studies.

3) Significant results or key outcomes:

Hynes lab:

ECM Proteomics
We have worked with the newly established proteomics facility at the Koch Institute to implement the methods we developed with the Proteomics Platform at the Broad Institute including direct comparisons of samples in both facilities and establishment of the bioinformatics pipeline worked out with the Broad collaborators. We therefore now have access to two proteomics facilities allowing faster turnaround of samples than previously. We have also implemented new TMT (using isobaric **t**andem **m**ass **t**ags) for quantitative comparisons among

samples and we are now using 10-plex comparisons, which significantly increases sample throughput and reduces processing time and expense.
We have prepared 3 independent sets of metastases to three different sites (lung, liver, brain, bone marrow) from implants of human MDA-MB231 cells into mice and prepared ECM-enriched (matrisome) preparations from them. These are about to be analyzed by 10-plex TMT quantitative proteomics to identify site-specific differences in the ECM microenvironment in different metastatic niches.

PDX models:
We have prepared ECM-enriched fractions from four PDX models of triple-negative mammary cancer differing in metastatic potential (tumors provided by the Polyak lab) and validated the enrichment obtained. These samples will now be subjected to our mass spec proteomic approaches to analyze and compare their ECM compositions. We will add additional PDX samples to this set in ongoing work.

Breast tissue samples:
We have also obtained human triple-negative breast cancer samples – both primaries and metastases through collaborations with the Polyak laboratory and their contacts. These will be subjected to analyses in parallel with the PDX models described above. Our goal will be to compare in detail the results obtained and published (Naba et al, 2013) using orthotopic tumors of the human mammary cancer cell line, MDA-MB231, with those from PDX models and patient samples – all derived from triple-negative tumors. These comparisons will provide a firm basis for selection of ECM biomarkers of metastasis, going beyond those we established in our earlier published work.

Investigation of Sned1 functions in knockout mice
One of the markers that we showed using the MB231 model as an functional enhancer of metastasis, particularly of malignancy and invasion at the primary site, is a large ECM protein known as Sned1. When we initially obtained these results, very little was known about this protein but our analysis of published RNA expression data showed that overexpression of Sned1 correlates with reduced survival of patients with ER-PR- mammary cancer, conforming well with our functional evidence for a causal role in the mouse model. Subsequently, other data have accumulated, further implicating this protein in tumor progression and metastasis. In order to investigate further its functions, we generated a mouse knockout model to explore its normal functions. The knock-out allele includes a LacZ transgene allowing facile detection of the expression pattern of the gene. During development, it is expressed in discrete patterns especially in tissues undergoing an epithelial-mesenchymal transition (EMT), such as neural crest and sclerotome (precursors of cartilage and bone), which is intriguing given the apparent role of Sned1 in invasion. However, it is not essential for this process since embryos progress through development and many are born, although at less than expected Mendelian ratios. There is some embryonic and significant peri-natal mortality and the surviving mice are small and show defects in bone density and in morphology of many bones, especially crano-facial (neural-crest-derived). We are continuing with these analyses to elucidate the normal roles of Sned1 with a view to understanding its ability to enhance invasion and metastasis. Sned1 may also play a role in ECM organization since tumors lacking Sned1 have very differently organized collagen. We have conducted TMT proteomic comparisons between tumors expressing and lacking Sned1

and we will also compare WT and KO tissues from the mice. We expect these analyses will provide insights into the role of Sned1 in the recruitment of other proteins to the matrix for use in our proposed aims for subsequent investigation – see list above.

Validation and generation of anti-ECM antibodies

We have been testing and validating antibodies against ECM proteins detected as being upregulated in malignant tumors and metastases in our published proteomic analyses. Many commercially available antibodies are clearly inadequate and we have been relaying our data back to the companies in an attempt to improve the information available to others using these antibodies. However, we have validated a panel of reliable antibodies to ECM proteins of interest and have established a good pipeline for preparation, immunohistochemical staining and pathological evaluation of tumor samples and we are using those to explore possible useful biomarkers. Nonetheless, we have need of additional antibodies and expect to discover other potential ECM biomarkers in our ongoing studies. Thus, we have begun a project to use camelid single-domain antibodies for these and other studies and have been experimenting with alpacas. In studies supported by another grant we have immunized two animals with [1] a cocktail of ECM proteins as well as peptides for others that we could not obtain in sufficient amounts; [2] matrisome preparations of colon cancer metastases. Both animals gave excellent immune responses and we have prepared a library of single Ig domain from one of them and begun screening for useful antibodies. These results serve as very encouraging proof-of-concept studies for our goals under this grant, obviously targeted against mammary cancer. We plan to screen these libraries for antibodies against ECM proteins of interest for breast cancer. We are also about to immunize two additional animals with matrisome preparations from human breast cancer metastases to lung and liver. We expect these experiments to generate a large number of monoclonal single-domain antibodies to ECM proteins of interest relative to metastasis.

Polyak lab:

Breast tissue samples:

In the first year of the grant we have collected 35 primary invasive tumors, 6 metastases (including 6 brain mets), and 6 pleural effusions. These fresh tissue samples were used for PDX generation and from some that had high cell numbers we have also tried to establish primary cultures (succeeded with 2-3 of pleural effusions). Most of these tumors were triple-negative breast cancer (TNBC) and a few inflammatory breast cancers (IBC), since these tumors are highly metastatic and currently have limited therapeutic options besides chemotherapy.

PDX models:

For establishment of new PDX models, we have injected dissociated tumor cells into the mammary fat pad of 6 weeks old NOG mice, 2 injecion/mouse, 2 mice/tumor for first passage followed by expansion to 5-10 mice/tumor. Several of these PDX tumors take months to grow (some >6 months), thus, each round of growth/expansion takes a few months.

We have generated these new PDX models in the past year: PE20 (TNBC, IBC), PE21 (TNBC, ILC), PE23 (TNBC, IBC), PE24, PE26 (TNBC, IBC, diffusely metastatic), PE28 (ILC), T112 (ER+, lung mets), T160 (TNBC, lung mets), T179 (TNBC, no mets), T272 (TNBC, no mets).

We have expanded (or in the process of expanding) HCI-001 (metastatic TNBC), HCI-002 (non-metastatic TNBC), IDC50-X (TNBC, highly locally invasive), and T272X (TNBC, non-

metastatic), HCI-009 (TNBC), and HCI-004 (TNBC). We repeated injections for all of them except T272X because of the concern about Matrigel (which we usually use for these injections to improve the efficiency of tumor growth). We have also obtained five additional PDX models that have been extensively characterized for molecular features and therapeutic responses (both in the patient and in mice) and we plan to expand these also in the upcoming year.

Cell line models of metastatic breast cancer:
We have injected 6 weeks old NOG mice mammary fat pads with derivatives of MDA-MB-468 breast cancer cell line at 500,000 cells per site (two injections/mouse, 5 mice/group). The primary tumors were surgically removed about 6-7 weeks post injection and snap frozen. We had several groups of tumors for this experiment: Mixed polyclonal tumors (these are metastatic): these tumors has an initial composition at the time of injection of about 10% IL11 GFP, 10% FIGF M-cherry, 40% parental CFP and 40% parental CD90.1. Control monoclonal tumors (these are rarely metastatic): 100% IL11 tumors, 100% FIGF tumors, 100% Parental CFP, 100% Parental CD90.1. We have also noted a difference in the metastatic behavior of these tumors when injected into NCR nude or NOG mice: we have observed higher frequencies of metastases in NOG mice but the primary tumors also had different phenotypes with the tumors being more "spongy" in the nude compared to NOG mice. Because some of these differences may be due to strain-specific differences in ECM proteins relevant to metastases, we are planning to analyze the same tumors injected into these two different strains of mice.

Key research accomplishments

A major focus of this project is to test the hypothesis that the ECM composition of primary tumors may predict metastatic behavior and to identify and characterize ECM markers that can be used for the detection of metastatic lesions at an early (small) stage and as novel therapeutic targets in breast cancer.

In the first year of this project we have made excellent progress on our goals for the initial year of support by this grant. We have established a productive collaborative effort between the two laboratories involved - Hynes (MIT) and Polyak (DFCI). We have collected and processed tissue from primary breast tumor samples and distant metastatic lesions of human patients, PDX models and xenografts of cell line models of breast cancer, including metastases to several different sites, both from xenograft models and from patients. We have already analyzed some of these by ECM-targeted proteomics and will analyze the rest in the near future. These results will establish a firm basis for identifying ECM biomarkers of significance.
We have already characterized a panel of antibodies to ECM proteins of interest and are well on the way to generating many more using novel single-domain antibody generation methods.
We have also pursued investigations of the functional roles of several ECM proteins that we earlier identified as enhancers of metastasis, most particularly one of them, Sned1, for which we have built a genetically engineered mouse to investigate its functions.

4) Other achievements:

None

What opportunities for training and professional development has the project provided?

The project does not have training as a specific goal. However, the professional development of the postdoctoral fellows and research scientists working on the research is an intrinsic part and implicit goal of the research project. These people learn a great deal, both in terms of specific experimental methods and strategic approaches in the course of their research. They learn from and train each other and also receive a great deal of training from experts in the shared research facilities with which they work (proteomics, bioinformatics, microscopy, animal imaging, histology, pathology) as well as from collaborators, most especially the interactions between the Hynes and Polyak laboratories. They also attend scientific meetings sduring the year in order to keep abreast of the filed and present and discuss their own work. There are also numerous relevant seminars and lectures in the MIT and HMS communities that provide further broadening of experience. All these inputs contribute in important ways to the development of these scientists and preparation for their individual careers.

How were the results disseminated to communities of interest?

Results are disseminated through presentations at meetings and seminars both in the local community and elsewhere, both in the USA and overseas. Our publications and web sites provide open access to all interested parties. We have also met with the breast cancer advocates at DFCI to inform them of our project and its goals and intend to repeat those meetings at appropriate intervals.

What do you plan to do during the next reporting period to accomplish the goals?

We will build on the results, methods and samples that we have already established. We are well on track with our initial proposed timetable – even slightly ahead in terms of antibody generation. We will continue to collect patient samples, focusing on large primary tumors and distant metastatic lesions, and also establish and characterize additional PDX models from these and conduct additional proteomic analyses to establish further the ECM proteins of most interest to pursue. We will continue our analyses of the functional roles of ECM proteins identified as well as others we expect to identify in the course of our investigations. We will also pursue the analyses of differences among the metastatic niches at different distant sites and, in a collaboration arising from discussions between our two laboratories, we will compare the heterogeneity of primary tumors both at the genetic and molecular marker level (Polyak) in parallel with analyses of ECM heterogeneity across the same tumors (Hynes)
We will exploit our initial forays into the generation of alpaca-derived single-domain monoclonal antibodies to ECM proteins. These antibodies are very stable, low molecular weight (15kDa) and facile to engineer with added tags for fluorescence and other imaging modalities including nanoparticles and we expect to be able to collaborate with local colleagues expert in such approaches to use these reagents for imaging and, in later years, for targeting metastases and recurrent primary tumors. Meanwhile we will also continue to explore the use of antibodies for diagnostic and prognostic approaches.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our focus on the nature of the tumor ECM microenvironment and its changes in metastasis is novel and the collaboration between our two complementary laboratories allows exploration of novel questions in breast cancer metastasis, including the impact of tumor heterogeneity.
The methods and approaches we have developed and are further developing are increasingly being used by others to investigate tumor progression and metastasis and, we believe, will contribute to future advances in understanding and combatting this most challenging aspect of cancer

What was the impact on other disciplines?

The technology we have developed and are continuing to further develop for detailed analyses of ECM composition and changes therein is already beginning to be applied to other diseases beyond cancer, such as fibrotic diseases and will likely have impact there.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

One of the lead postdoctoral fellows obtained fellowship support for some of the period, so those salary costs were underutilized and will be used to hire another technical assistant.

Some of the microscope upgrades budgeted were held up by production delays at the manufacturers – we expected those upgrades to be delivered within year 1 but they have been delayed to year 2.

Since our ACURO requests for use of alpacas for immunization were twice rejected, we elected to proceed with those experiments using other funds. The funds initially assigned for those experiments will be deployed as soon as approval is obtained and are being used for the downstream processing of lymphoblasts obtained using other non-DoD sources of funding.

All three of those delays plus others (now resolved) in obtaining initial approval of our mouse and human subject protocols meant that our spending in year 1 was somewhat lower than expected but we anticipate that all those costs will be incurred in year 2.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Apart from the delays in approvals mentioned above, there are no other significant changes.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Hynes lab mouse protocol renewal was approved by the MIT Institutional Animal Care and Use Committee for period 4/17/15-4/17/18 and approved by the DoD ACURO on July 30. 2015

Polyak Lab Animal Study reviewed and approved by Institutional Animal Care and Use Committee for period 10/18/14-10/18/15 – the new renewal was already submitted and expected to be approved in early October.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to report as yet but see below

  Books or other non-periodical, one-time publications.

  Nothing to report as yet but see below

  Other publications, conference papers, and presentations.

  The publications listed below, although based on work prior to this award, all concern the research on which this award and research supported by it and serve as resources for the breast cancer research community along with the web site described below.

  1. Naba, A., Clauser, K.R., Lamar, J.M., Carr, S.A. and Hynes, R.O. (2014a). Extracellular Matrix Signatures of Human Mammary Carcinoma Identify Novel Metastasis Promoters. eLife 2014;3:e01308. DOI: 10.7554/eLife.01308; PMID:24618895 PMC3944437.

  2. Labelle, M., Begum, S. and Hynes, R.O. (2014). Platelets guide the formation of early metastatic niches. Proc. Natl. Acad. Sci. USA, 2014 Jul 29;111(30):E3053-61. doi: 10.1073/pnas.1411082111. Epub 2014 Jul 14. PMID:25024172 PMC4121772

  3. Naba, A., Clauser, K.R., Carr, S.A., Tanabe, K.K. and Hynes, R.O. (2014b). Extracellular Matrix Signatures of Human Primary Metastatic Colon Cancers and their Liver Metastases. BMC Cancer. 2014 Jul 18;14(1):518. [Epub ahead of print] PMID:25037231 PMC4223627

  4. Naba, A., Clauser, K.R., Ding, H., Whittaker, C.A., Carr, S.A. and Hynes, R.O. (2015a). The extracellular matrix: tools and insights for the "omics" era. Matrix Biol. 2015 Jul 7. pii: S0945-053X(15)00121-3. doi: 10.1016/j.matbio.2015.06.003. PMID:26163349

  5. Naba, A., Clauser, K.R. and Hynes, R.O. (2015b). Enrichment of extracellular matrix proteins from tissues and digestion into peptides for mass spectrometry analysis. J Vis Exp. (JoVE) 2015 Jul 23;(101). doi: 10.3791/53057. PMID:26273955

Lectures, Symposia - Hynes

Whitehead Institute/MD Anderson Symposium on Hallmarks of Cancer, Oct.24, 2014
"Tumor Microenvironment: Extrinsic Contributions to the Steps of Metastasis"
Salvador Luria Lecture, MIT, Nov. 18, 2014
"Tumor Cells Have Lots of Help During Metastasis"
Svedberg/Rudbeck Lecture ,Uppsala University, Sweden, Feb. 9, 2015
"Extracellular Matrix and Platelets: Extrinsic Enhancers of Metastasis"
TCB/Wihuri Special Seminar, Biomedicum, Helsinki, Finland, Feb. 10, 2015
"Extracellular Matrix and Platelets: Extrinsic Enhancers of Metastasis"
Nobel Forum Lecture, Karolinska Institute, Stockholm, Sweden, Feb. 11, 2015
"Extracellular Matrix and Platelets: Extrinsic Enhancers of Metastasis"
Univ. Massachusetts, Amherst, May 15, 2015
"Tumor Cells Have Lots of Help During Metastasis"

- **Website(s) or other Internet site(s)**

We have developed (using other funding) a web site <http://hynes-lab.mit.edu/matrisomeproject> detailing our methods for proteomic and bioinformatic analyses of extracellular matrix (ECM) proteins (also reported in publications 4 & 5 above). This is an interactive hyperlinked resource to enable other workers to deploy our methods. The data and information presented there include but are not limited to breast cancer research and this site is proving to be increasingly used as a resource by other researchers.
We will maintain and update this site, including the data developed under this award.

- **Technologies or techniques**

Described in publications and on web site listed above

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Again, see list of publications, especially #s 4 and 5 and web site cited above.
We also routinely supply mouse models and cell lines, recombinant DNA probes and constructs and antibodies to other researchers.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Koch Institute, MIT

Where individuals are listed for less than 12 months that is because some of their effort was dedicated to other projects and supported by other funds.

Name: Richard Hynes
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-7603-8396
Nearest person month worked: 6

Name: Alexandra Naba
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID): 0000-0002-4796-5614
Nearest person month worked: 7
Contribution to Project: Developer of the proteomics methods deployed in the project. Analysis of identified ECM proteins enhancing metastasis, including development of genetically engineered mice.

Name: John Lamar
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID): 0000-0003-0547-3619
Nearest person month worked: 5
Contribution to Project: Provided expertise, reagents and experimental support for xenotransplants and retroviral and lentiviral modulations of gene expression.

Name: Noor Jailkhani
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-4933-1068
Nearest person month worked: 12
Supported by Postdoctoral Fellowship
Contribution to Project: Conducted proteomic analyses of ECM-enriched (matrisome) preparations from PDX models and human patient samples. Initiated generation of recombinant single-chain antibodies to ECM proteins of interest.

Name: Steffen Rickelt
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-5224-7764
Nearest person month worked: 4
Contribution to Project: Developed and validated antibodies and immunohistochemical methods for assessing in situ expression of ECM proteins of interest.

Name: Jess Hebert
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 0000-0002-8778-5941
Nearest person month worked: 6
Additional Support from MIT Biology Department Training Grant
Contribution to Project: Developed methods for generating and harvesting mammary carcinoma metastases to diverse sites (lung, liver, brain, bone marrow) and collected sufficient well characterized samples form all these sites to proceed to proteomic analyses of matrisome preparations from these sites (in progress).

Name: Ying Huang
Project Role: Technical Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6
Contribution to Project: Assisted with many aspects of the research led by Research Scientists and Postdocorals.

DFCI Sub-contract

Name: Kornelia Polyak
Project Role: Partnering PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-5964-0382
Nearest person month worked: 1

Contribution to Project: Dr. Polyak has supervised the project in her lab and coordinated collaboration with the Lindquist lab.
Funding Support: Please see previously provided other support and changes noted below.

Name: Doris Tabassum
Project Role: Graduate student
Researcher Identifier (e.g. ORCID ID): 0000-0002-4830-7146
Nearest person month worked: 3

Contribution to Project: Doris Tabassum has generated cell line models of heterogeneity with different metastatic capability. Additional funds from Dr. Polyak's grants from the DOD and Breast Cancer Research Foundation.

Name: Guillermo Peluffo (Polyak lab)
Project Role: research fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-9855-3986
Nearest person month worked: 6
Contribution to Project: Dr. Peluffo has been generating and expanding PDX models for the study. He has also been handling the fresh primary patient breast tissue collection and processing for the lab.
Funding Support: Additional funds from Dr. Polyak's grants from Novartis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Changes in Richard Hynes' other support:

Grants ended (July 2014 – present)

1) Breast Cancer Alliance (PI Hynes).
   Development of diagnostic and prognostic approaches exploiting metastasis-specific ECM proteins
   01/01/14-12/31/14
2) NCI Integrative Cancer Biology Program (PI Lauffenburger)
   Regulatory Networks in Cancer Initiation and Progression
   3/1/10-2/28/15

No new grants awarded

Changes in Kornelia Polyak's other support:

Grants ended (June 2014 – present)

1) V Foundation - Translational Research Grant (Lin/Polyak)
   Targeting the Il-6/Jak/Stat3 pathway in human breast cancer
   08/01/2010 – 07/31/2014
2) Inflammatory Breast Cancer Research Foundation (Overmoyer/Polyak)
   Validation of Jak2 as a novel therapeutic target in triple negative inflammatory breast cancer
   07/1/2011 - 7/31/2014
3) Breast Cancer Alliance - Exceptional Project Grant (Polyak)
   Microenvironment-induced metabolic alterations and therapeutic resistance in breast cancer
   01/01/2014-12/31/2014
4) Susan G. Komen Foundation-KG110127 (Polyak)
   The Molecular Basis of Breast Density and its Implications for Breast Cancer Prevention
   08/30/2011 – 08/29/2014
5) NIH/NCI U01 CA143233 (Polyak)
   Myoepithelial cell differentiation defects in ductal carcinoma in situ (DCIS)
   09/30/2009 – 08/31/2014
6) Novartis-DFCI Drug Discovery Program (Polyak)
   JMJD2C histone demethylase as therapeutic target in breast cancer
   01/01/2013 – 12/31/2014

New grants awarded: **Grants beginning (June 2014 – present)**

U01 CA195469 (Polyak/Michor/Spellman/Gray) 06/01/15 – 05/31/20 NIH/NCI — 0.60 Calendar Months (5% Effort)

Role: Principal Investigator

Intratumor heterogeneity underlying treatment resistance in HER2+ breast tumors

Specific Aims: 1) Develop a multi-scale model of primary and metastatic breast tumors; Aim 2) Parameterize the multi-scale mathematical model based on data from mouse xenograft models; Aim 3) Use the multi-scale model to predict disease kinetics and optimum prevention and treatment strategies, and validate these strategies in mouse xenograft models.
POC: Rebecca Brightful-Grants Management Specialist; Email: brightfr@mail.nih.gov; Phone: 301-631-3011

U54 CA193461 (Michor) 05/19/15 – 04/30/20 0.60 Calendar Months
NIH/NCI (5% Effort)
Role: Project 3 Principal Investigator
Evolution and Treatment Response of Brain, Breast, and Hematologic Malignancies – Project 3
Single Cell Measures of Intratumor Diversity for Optimal Breast Cancer Therapy
The Dana-Farber Cancer Institute-Physical Sciences-Oncology Center (DFCI-PSOC) brings together a trans-disciplinary research team to advance understanding of the physical principles that govern the response of tumor cell populations to treatment and the emergence of resistance.
Specific Aims – Project 3: 1) Perform single cell analyses of breast tumor samples; (2) Characterize therapeutic responses in xenograft models of breast cancer; and 3) Predict optimal therapeutic strategies to prevent metastatic outgrowth and treatment resistance and validate these strategies in xenograft models.
POC: Rebecca Brightful-Grants Management Specialist; Email: brightfr@mail.nih.gov; Phone: 301-631-3011

W81XWH-14-1-0212 (Polyak/Meissner) 09/30/14 – 09/29/16 0.60 Calendar Months
BCRP Idea Expansion Award: Collaborative 5% Effort
Role: Principal Investigator
Epigenetic Subtypes of Triple-Negative Breast Cancer
Specific Aims: 1) Define epigenetic heterogeneity in TNBCs; and 2) Explore the role for histone demethylases in epigenetic heterogeneity in TNBCs.
POC: Contract Specialist: Cheryl A. Lowery, Email; cheryl.a.lowery8.civ@mail.mil; Phone: (301) 619-7150

W81XWH-14-1-0158 (Polyak/Lindquist) 06/01/2014 – 05/31/2017 0.24 Calendar Months
Breast Cancer Research - Breakthrough
Role: Principal Investigator
HSF1 Enables the Evolution of Aggressive Breast Cancers
Specific Tasks: 1) Define patient populations and therapeutic settings in which HSF1 activation correlates with greater intratumor heterogeneity and, consequently, a higher risk of metastatic progression and therapeutic resistance; 2) Evaluate activation of HSF1 in a cell line-derived xenograft model, and in transplantable patient-derived xenografts, and correlate with intratumor heterogeneity and metastasis; 3) Test the effect of inhibiting HSF1, HSP90 or both on intratumor heterogeneity and metastatic potential of patient-derived tumors in mice; 4) Study the emergence of drug-resistance in xenograft models; and 5) Complete experiments, analyze data and submit it for publication.
POC: Contract Specialist: Cheryl A. Lowery, Email; cheryl.a.lowery8.civ@mail.mil; Phone: (301) 619-7150

Breast Cancer Research Foundation (Polyak) 10/02/08 – 09/30/15 1.80 Calendar Months
Innovative Research Grant (15% Effort)
Role: Principal Investigator
Molecular basis of breast tumor heterogeneity and its clinical consequences
The major goal of this grant is to determine the mechanisms underlying intra-tumor heterogeneity and their clinical relevance.
POC: Deputy Director: Margaret Mastrianni Email: mailto:pegmast@bcrfcure.org Phone: (646) 497-2600

Breast Cancer Research Foundation (Polyak) 10/02/08 – 09/30/16 1.80 Calendar Months
Innovative Research Grant (15% Effort)
Role: Principal Investigator
Molecular basis of breast tumor heterogeneity and its clinical consequences
The major goal of this grant is to determine the mechanisms underlying intra-tumor heterogeneity and their clinical relevance.
POC: Deputy Director: Margaret Mastrianni Email: mailto:pegmast@bcrfcure.org Phone: (646) 497-2600

(Polyak) 01/01/15 – 12/31/17 0.12 Calendar Months
DFCI-NOVARTIS Drug Discovery Program (1% Effort)
Role: Principal Investigator
Integrated analysis of heterogeneity in and drivers of metastatic cancers
Specific Aims: 1) Determine the contribution of genetic heterogeneity to metastasis in breast cancer; 2) Develop strategies to assess genomic heterogeneity in human tumors; and 3) Develop methods to generate models of metastatic tumors.
POC: Program Administrator Sylvia C. Lin Email**:** Sylvia_Lin@dfci.harvard.edu Phone**:** (617) 632-5599

(Polyak/Brown/Roberts/Shivdasani/Stegmaier)01/01/15 – 12/31/17 0.12 Calendar Months
DFCI-NOVARTIS Drug Discovery Program (1% Effort)
Role: Principal Investigator
Epigenetic dependencies in human cancer
Specific Aims: 1) Identify changes in epigenetic dependencies following pharmacologic perturbations; 2) Identify dependencies of cancer cells resistant to epigenetic modulators; and 3) Perform a genome-wide CRISPR screen to investigate mechanisms of resistance to epigenetic therapies.
POC: Program Administrator Sylvia C. Lin Email**:** Sylvia_Lin@dfci.harvard.edu Phone**:** (617) 632-5599

Ludwig Center at Harvard (Brugge, Demetri) 0.10 Calendar Months
04/01/15-03/31/16 Ludwig Center (1% Effort)
Role: Principal Investigator
Epigenetic heterogeneity in breast cancer
POC: Jane Staunton, PhD-Director of Scientific Administration and Education; Email: jane_staunton@hms.harvard.edu; Phone: 617-432-5920

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A

References N/A